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54 **Composition for increasing the quantity and quality of fruits and flowers of plants.**

57 **A composition suitable for increasing the quantity and quality of fruits or flowers of plants in horticulture and agriculture, which comprises**

- (A) at least one 24-epibrassinolide, and
- (B) at least one nontoxic salt of choline;

and a method of increasing the quantity and quality of fruits or flowers of a plant in horticulture and agriculture, which comprises applying a fruit or flower quantity and quality improving effective amount of (A) at least one 24-epibrassinolide and (B) at least one nontoxic salt of choline to the plant or its cultivated area.

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This invention relates to a composition and a method for increasing the quantity, and/or improving the quality, of fruits or flowers of plants in horticulture and agriculture.

5 Five types of compound, auxins, gibberellins, cytokinins, abscisic acids and ethylenes, have previously been known as substances considered to be phytohormones. The gibberellins have practical effects and are used actually in crop cultivation as a plant growth promoter; 10 the auxins such as indoleacetic acid and alpha-naphthaleneacetic acid, as a plant root growth promoter; and the cytokinins such as kinetin (6-furfurylaminopurine) and benzyladenine, as a plant aging inhibitor.

Recently, brassinolide, a kind of steroid, was 15 isolated from an extract of the pollen of Brassica napas as a new phytohormone, and its chemical structure was determined [see Michael D. Grove et al., Nature, 281, 20 Sept. pp. 216-217, (1979)]. Since then, ten and several brassinolide-like steroids having biological activity on 20 plants have been found in various plants, but their possibility of utilization in an agricultural field is still on the stage of research and experiment.

On the other hand, choline salts, a group of vitamin B compounds, have been widely used as animal feed 25 additives. U. S. Patent No. 4,309,205 recently disclosed that non-toxic choline salts enhance the reproductive development of plants. Their enhancing effect, however, is not entirely satisfactory for practical purposes.

It has now been found in accordance with this 30 invention that a combination of a 24-epibrassinolide and a choline salt shows a synergistically high effect for increasing the quantity, and/or improving the quality, of fruits or flowers of plants as compared with the use of

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the individual components of the combination.

The present invention provides a composition suitable for increasing the quantity and quality of fruits or flowers of plants in horticulture and agriculture, which comprises

(A) at least one 24-epibrassinolide, and

(B) at least one nontoxic salt of choline.

The 24-epibrassinolide used as one active ingredient in the composition of this invention includes (22R,23R,24R)-2 α ,3 α ,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5 α -cholestan-6-one (to be sometimes abbreviated hereinafter as "22R,23R-Epi BR"), and (22S,23S,24R)-2 α ,3 α ,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5 α -cholestan-6-one (to be sometimes abbreviated hereinafter as "22S,23S-Epi BR"). They may be used either singly or in combination. These 24-epibrassinolides are known compounds and can be synthesized, for example, by the methods described in M. J. Thompson et al., J. Org. Chem. 44, 5002 (1979); Steroids, 39, 89 (1982); and K. Mori et al., Agricultural Biological Chemistry, 44 (5), 1211-1212 (1980), *ibid.*, 47, 663 (1983), *ibid.*; 47, 925 (1983).

The nontoxic choline salt, the other active ingredient used in combination with the 24-epibrassinolide in the composition of this invention, includes organic or inorganic salts of choline which are not phytotoxic, nor toxic to humans and animals in the concentrations in which they are used. Specific examples include inorganic salts of choline, such as choline hydrochloride, dihydrogen phosphate, polyphosphate, sulfate, nitrate, silicate or carbonate; and organic salts of choline such as choline acetate, dihydrogen citrate, lactate or hydrogen L(+)-tartrate. These salts may be used singly or in combination.

The composition of this invention may be formulated into any desired form such as a dust, granules, a wettable power, a solution, a suspension, an emulsifiable

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concentrate and an aerosol according to the method of applying the composition. The composition may be formulated by procedures known per se in the art. For example, the dust, granules or wettable powder can be prepared by mixing and pulverizing at least one 24-epibrassinolide, at least one choline salt and at least one solid carrier or diluent of the types described above, adding a suitable amount of a surface-active agent, and mixing them uniformly. The solution, suspension or emulsifiable concentrate may be prepared by dissolving or dispersing at least one 24-epibrassinolide and at least one choline salt in at least one liquid carrier or diluent, and if desired, adding a suitable surface-active agent.

The agronomically acceptable carrier or diluent means any substance which can be used to dissolve, disperse or diffuse the active components in the composition without impairing their effect of increasing the quantity and/or quality of fruits or flowers of plants, and which by itself has no detrimental effect on the soil, equipment, crops, or an agronomic environment.

Examples of solid carriers useful in the composition of this invention include vegetable powders (e.g., starch, and acacia), mineral powders, clay minerals (e.g., kaolinite group, and montmorillonite group), talc, pyrophyllite, vermiculite, calcite, gypsum, silica gel, mica group, dolomite, magnesite, kieselguhr, slaked lime, pumice, sulfur, inorganic salts (e.g., calcium carbonate), and synthetic polymers (e.g., phenolic resin or urea resin). Examples of liquid carriers or solvents which are useful in the composition of this invention include water, alcohols (e.g., methanol, ethanol, propanol or ethylene glycol), ketones (e.g., acetone, methyl ethyl ketone or methyl isobutyl ketone), amides (e.g., N,N-dimethylformamide or N,N-dimethylacetamide), and sulfoxides (e.g., dimethyl sulfoxide).

Suitable emulsifiers include, for example, ethylene oxide derivatives of alkylphenols or long-chain alcohols, mercaptans, carboxylic acids, reactive amines, and partially esterified polyhydric alcohols. Solvent-
5 soluble sulfates or sulfonates, such as alkaline earth metal salts or amine salts of alkylbenzenesulfonates and aliphatic alcohol sodium sulfates, having surface-active properties can be used as emulsifiers either singly or in combination with an ethylene oxide reaction product.

10 The composition of this invention comprises a mixture of the 24-epibrassinolide and the choline salt in an amount effective for increasing the quantity and quality of fruits and flowers. The specific amount of the mixture may vary depending upon the form of the
15 composition. Generally, the composition of the invention may contain at least 2% by weight, preferably 20 to 80% by weight, more preferably 30 to 50% by weight, of the mixture of the 24-epibrassinolide and the choline salt based on the weight of the composition.

20 The dust or granules may contain the mixture of the 24-epibrassinolide and choline salt in a concentration of 2 to 75% by weight based on the weight of the composition, and the wettable powder, solution, suspension or emulsifiable concentrate may contain 10 to 75% by weight
25 of the mixture.

The composition of this invention may also contain a fungicide, a bactericide, an insecticide, an acaricide, a nematocide, a fertilizer, a herbicide and other plant growth regulating agent, which are usually
30 employed in agriculture.

The proportions of the 24-epibrassinolide and the choline salt in the composition of this invention should not be limited to a narrow range, and may be varied over a broad range depending, for example, upon
35 the type or the growth stage of a plant to which the composition is to be applied. Generally, the suitable

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weight ratio of the 24-epibrassinolide to the choline salt is from 1:100 to 1:10¹⁰, preferably from 1:1,000 to 1:10⁹, more preferably from 1:3,000 to 1:10⁸.

Preferred forms of the composition of this invention are a wettable powder and a dilute or concentrated aqueous solution or dispersion which are to be used by dilution with an aqueous medium.

The formulation containing the 24-epibrassinolide and the choline salt described above can be applied to plants after it is diluted with a suitable diluent such as water so that the concentration of the 24-epibrassinolide falls generally within the range of 0.001 ppb to 3 ppm, preferably within the range of 0.01 ppb to 1 ppm, and the concentration of the choline salt falls generally within the range of 5 to 3,000 ppm, preferably within the range of 20 to 2,000 ppm.

The following Formulation Examples illustrate the formulation of the composition of this invention.

FORMULATION EXAMPLE 1

Wettable powder:-

<u>Ingredient</u>	<u>Parts by weight</u>
22S,23S-Epi BR	0.01
Choline dihydrogen phosphate	30
Sodium dodecylbenzenesulfonate	5
Polyoxyethylene alkylphenyl ether	1
Talc	63.99

The above ingredients are uniformly mixed and pulverized in a mill. The resulting wettable powder can be used generally after diluting it with water to 10 to 200 times.

FORMULATION EXAMPLE 2

Aqueous solution:-

<u>Ingredient</u>	<u>Parts by weight</u>
22S,23S-Epi BR	0.001
75% aqueous solution of choline chloride	32
Polyoxyalkylaryl ether	2
Laurylmethyldihydroxyethyl ammonium chloride	1
i-Propyl alcohol	33
Water	31.999

The above ingredients are uniformly mixed to form an aqueous solution.

The 24-epibrassinolide and choline salt in the composition of this invention may be taken into a plant from its roots or leaves by applying it through a soil treatment, for example adding the composition to irrigating water and directly applying it to the soil, or through a foliar treatment by directly spraying it to the plant's stalks and leaves.

Thus, according to this invention, there is also provided a method of increasing the quantity and quality of fruits or flowers of a plant in horticulture and agriculture, which comprises applying a fruit or flower quantity and quality improving effective amount of (A) at least one 24-epibrassinolide and (B) at least one nontoxic salt of choline to the plant or its cultivated area.

Application of the 24-epibrassinolide and choline salt to plants in accordance with this invention may usually be via seeds by immersion, the root system by irrigation, or foliage by spraying. The rates of application of the 24-epibrassinolide and choline salt can be varied widely depending upon the kind and growth stage of the plant, the formulation, etc. Generally, when the composition is applied via seeds by immersion or the root

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system by irrigation, the suitable concentrations of the 24-epibrassinolide and the choline salt in an aqueous solution are 0.03 ppb to 0.1 ppm, and 10 to 1,000 ppm, respectively, for rice seedlings, and 0.001 to 1 ppm and 50 to 300 ppm, respectively, for tomato. In the case of foliar application to rice, wheat and barley, the composition of this invention is sprayed after diluting it with 10 to 1,000 liters/ha of water so as to provide a 24-epibrassinolide concentration of 0.1 ppb to 10 ppm and a choline salt concentration of 500 ppm to 1%. For foliar application to corn, the composition may be sprayed after being diluted to 10 to 1,000 liters/ha of water so as to provide a 24-epibrassinolide concentration of 0.1 ppb to 0.01 ppm and a choline salt concentration of 1000 ppm to 1%.

There is no particular limitation on the time of applying the composition of this invention. It may be applied at any desired stage of plant growth whereby the growth of roots, stalks and leaves of plants can be promoted and the quantity and/or quality of flowers and fruits can be increased or improved. Especially preferably, the composition of this invention is applied to plants in their reproductive stage. The time of application at which the greatest effect can be obtained varies depending upon the kind of the plant, and any skilled person in the art can easily determine the best time by performing routine experiments for each of the plants to which the composition of this invention is to be applied. For example, to rice, barley and wheat, it is preferable to apply the composition of the invention during the time from about 40 days before the flowering time to 20 days after it, particularly from about 20 days before flowering to a time immediately after it.

The composition and method of this invention are applicable to a great variety of plants. Specific examples of such plants include cereal plants such as

rice, wheat, barley, and corn; leguminous plants such as soybean; plants having underground tubers or bulbs such as onion, garlic and potato; vegetables grown for their edible roots such as beet and carrot; fruits such as
5 peach, persimmon, grape and apple; vegetables grown for their edible fruits such as tomato and cucumber; vegetables grown for their edible leaves such as lettuce, cabbage, cauliflower and spinach; and flowers such as tulip and cosmos. In particular, the composition of this
10 invention can be advantageously for rice, wheat, corn and soybean. The composition of this invention achieves an especially good effect on wheat and rice.

According to the composition and method of this invention described above, the various excellent bene-
15 ficial effects tabulated below can be obtained according to crops and the method of application.

Crop	Method of application	Time of application	Rate of application of the choline salt	Rate of application of the 24 epibrassinolide	Expected effect
rice, wheat, barley	foliar	from 40 days before heading to 10 days after heading	500-2000 g/ha	0.01-1000 mg/ha	increased harvest
soybean (leguminous plants)	foliar	from 10 days before flowering to 20 days after flowering	500-1000 g/ha	0.1-1000 mg/ha	increased harvest (increased number of pods)
onion, garlic tulip	foliar	early stage of bulb swelling	500-2000 g/ha	0.1-1000 mg/ha	bulb swelling
potato	foliar	early stage of tuber growth	50-1500 g/ha	10-1000 mg/ha	tuber growth and increased yield
peach, persimmon, grape, apple	foliar	flowering stage to 10 days before harvest	25-2000 g/ha	0.1-1000 mg/ha	size increase of fruits, increase of sweetness, maintenance of freshness
wheat, barley	foliar	2- to 3-leaf stage	50-1000 g/ha	0.01-1000 mg/ha	growth promotion

- to be continued -

(continued)

Crop	Method of application	Time of application	Rate of application of the choline salt	Rate of application of the 24 epibrassinolide	Expected effect
rice	seed treatment	immersed for 24 hours after immersion in water for 2 days	50-1000 ppm	0.001-1 ppm	promotion of growth and root formation
wheat, barley	seed treatment	immersed for 24 hours	10-1000 ppm	0.001-1 ppm	promotion of growth and root formation
tomato, lettuce	seed treatment	immersed for 24 hours	1-300 ppm	0.001-1 ppm	promotion of growth and root formation
rice seedling	irrigation	from the 2-leaf stage to the time before transplantation	5-500 mg /1800 cm ² (10-1000 ppm)	0.0005-0.05 mg /1800 cm ² (0.0001-0.1 ppm)	promotion of growth and root formation
tomato seedling	irrigation	seedling stage	1-100 mg/ seedling (10-1000 ppm)	0.0001-0.01 mg/ seedling (0.0001-0.1 ppm)	growth promotion

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The following Test Examples specifically illustrate the excellent beneficial effects of the composition of this invention.

TEST EXAMPLE 1

5 Test on rice in Petri dishes:-

In each run, 10 ml of an aqueous solution containing 22S,23S-Epi BR (diluted from 1% wettable powder) and/or choline chloride (to be referred to as "CC") in each of the concentrations indicated in Table 1 was added to a deep Petri dish having a diameter of 9 cm, and ten rice seeds (variety: "nihonbare") immediately after emergence were sown in it. The Petri dish was put in a phytotron kept at 25°C, and the rice was cultivated while occasionally supplying water in an amount corresponding to the consumed water. Twelve days later, the length of the root and the length of the overground portion were measured, and the results are shown in Table 1.

Table 1

Chemical and concentration (ppm)	Ratio to the untreated	Length of the root
Untreated	100	100
22S,23S-Epi BR (0.03)	106	113
" (0.1)	107	121
CC (50)	105	124
" (100)	107	121
22S,23S-Epi BR (0.03) + CC (50)	111	155
" (0.03) + CC (100)	113	156
" (0.1) + CC (50)	112	140
" (0.1) + CC (100)	115	143

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TEST EXAMPLE 2

Test in a wheat field:-

On October 28, autumn-sowing wheat (variety: "norin #61") was sown in a field in Utsunomiya-City, Tochigi-ken, Japan. In the heading stage (April 27), an aqueous solution containing 22R,23R-Epi BR (diluted from 1% wettable powder) and/or CC in each of the concentrations indicated in Table 2 was applied uniformly once to foliage at a rate of 10 liters/are by a sprayer. Water was sprayed at a rate of 10 liters/are to a un-treated area. On June 15, the wheat was harvested, and the yield (the amount of fruits) was measured. The results are shown in Table 2. The experiments were carried out through three replications for each area.

Table 2

Chemical and concentration (ppm)	Average yield (ratio to the untreated area)
Untreated area	100 (5210 kg/ha)
22S,23S-Epi BR (0.001)	110
" (0.01)	113
CC (500)	108
" (1000)	110
22R,23R-Epi BR (0.001) + CC (500)	122
" (0.001) + CC (1000)	135
" (0.01) + CC (500)	123
" (0.01) + CC (1000)	125

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TEST EXAMPLE 3

Test on rice in pots:-

Rice (variety: "nihonbare") was cultivated outdoors in Wagner pots (1/5000 are), and in the heading stage (July 30), an aqueous solution containing 22R,23R-Epi BR (diluted from 1% wettable powder) and/or CC was sprayed uniformly to foliage at a rate of 5 ml/pot by a sprayer. On September 24, the rice was harvested and the yield (the amount of hulled rice) was measured (4 pots constituted one area).

The results are shown in Table 3.

Table 3

Chemical and concentration (ppm)	Amount of hulled rice (ratio to the untreated area)
Untreated area	100 (29 g/pot)
22R,23R-Epi BR (0.003)	104
" (0.01)	106
CC (300)	100
" (1000)	102
22R,23R-Epi BR (0.003) + CC (300)	113
" (0.003) + CC (1000)	114
" (0.01) + CC (300)	115
" (0.01) + CC (1000)	116

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TEST EXAMPLE 4

Test on growth of corn:-

Corn (dent corn) was grown to the 2-leaf stage in a 1-liter plastic pot. An aqueous solution containing 22R,23R Epi BR or 22S,23S Epi BR (diluted from 1% wettable powder) and/or CC in each of the concentrations shown in Table 4 was uniformly sprayed onto the corn by a sprayer. Thereafter, the corn was cultivated in a glass greenhouse. Twenty-five days after the spraying, the overground portion of the corn was reaped, and its dry weight was measured. The results are shown in Table 4.

Table 4

Chemical and concentration (pm)	Dry weight of the over-ground portion (ratio to the untreated area)
Untreated area	100 (2.0 g/for each corn)
22S,23S-Epi BR (0.01)	105
" (0.1)	100
22R,23R-Epi BR (0.01)	110
" (0.1)	107
CC (300)	120
" (1000)	124
22S,23S-Epi BR (0.01) + CC (300)	158
" (0.01) + CC (1000)	160
22R,23R-Epi BR (0.01) + CC (300)	139
" (0.01) + CC (1000)	148

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TEST EXAMPLE 5

Test on soybean in a field:-

Soybean (variety: "tachisuzunari") was sown in a field in Utsunomiya-City, Tochigi-ken, Japan on June 26. On August 19 which was 10 days after the beginning of flowering, an aqueous solution containing a 1:1 mixture of 22S,23S-Epi BR and 22R,23R-Epi BR and/or choline phosphate (to be referred to as CP) in each of the concentrations indicated in Table 5 was sprayed to foliage at a rate of 10 liters/are. Water was sprayed to a non-treated area at a rate of 10 liters/are. On October 12, the soybean was harvested. The amount of 100 soybeans and the amount of fruits were measured. The results are shown in Table 5.

Table 5

Chemical and concentration (ppm)	Amount of 100 beans (ratio to the untreated area)	Amount of fruits (ratio to the untreated area)
Untreated area	100 (23.6g)	100 (3450Kg/ha)
22S,23S-Epi BR		
+22R,23R-Epi BR(1:1) (0.01)	100	107
" (0.03)	101	105
" (0.1)	102	108
CP (1000)	98	110
22S,23S-Epi BR		
+22R,23R-Epi BR(1:1) (0.01)+CP(1000)	102	124
" (0.03)+CP(1000)	100	133
" (0.1) +CP(1000)	99	127

What is claimed is:

1. A composition suitable for increasing the quantity and quality of fruits or flowers of plants in horticulture and agriculture, which comprises
 - (A) at least one 24-epibrassinolide, and
 - (B) at least one nontoxic salt of choline.
2. The composition of claim 1 wherein the weight ratio of the 24-epibrassinolide to the choline salt is from 1:100 to $1:10^{10}$.
3. The composition of claim 2 wherein the weight ratio of the 24-epibrassinolide to the choline salt is from 1:1,000 to $1:10^9$.
4. The composition of claim 1 wherein the 24-epibrassinolide is selected from (22R,23R,24R)-2 α ,3 α ,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5 α -cholestan-6-one and (22S,23S,24R)-2 α ,3 α ,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5 α -cholestan-6-one.
5. The composition of claim 1 wherein the nontoxic salt of choline is selected from choline hydrochloride, dihydrogen phosphate, polyphosphate, sulfate, nitrate, silicate, hydrogen carbonate, acetate, dihydrogen citrate, lactate and hydrogen L(+)-tartrate.
6. The composition of claim 1 which comprises a fruit or flower quantity and quality improving effective amount of (A) at least one 24-epibrassinolide and (B) at least one nontoxic salt of choline, and an agronomically acceptable carrier or diluent.
7. The composition of claim 6 which is in the form of a wettable powder, an emulsifiable concentrate, a dust, granules, an aerosol or a flowable emulsifiable concentrate.
8. The composition of claim 7 which is in the form of a wettable powder, or a dilute or concentrated aqueous solution or dispersion.
9. A method of increasing the quantity and quality of fruits or flowers of a plant in horticulture and

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agriculture, which comprises applying a fruit or flower quantity and quality improving effective amount of (A) at least one 24-epibrassinolide and (B) at least one nontoxic salt of choline to the plant or its cultivated area.

10. The method of claim 9 wherein the 24-epibrassinolide and the choline salt are applied to the plant via the root system by irrigation or foliage by spraying.

11. The method of claim 9 wherein the 2,4-epibrassinolide and the choline salt are sprayed to the foliage of the plant at a rate of 0.01 to 1,000 mg/ha and 25 to 2,000 g/ha, respectively.

12. The method of claim 9 wherein an aqueous solution containing 10 to 1,000 ppm of the choline salt and 0.03 ppb to 1 ppm of 2,4-epibrassinolide is applied to seeds of the plant by immersion or to the root system of the plant by irrigation.

13. The method of claim 9 wherein the plant is selected from cereal plants of the group consisting of rice, wheat, barley and corn; soybean; beet; plant having underground tubers or bulbs of the group consisting of onion, garlic and potato; vegetables of the group consisting of tomato, cucumber, lettuce, cabbage, cauliflower, spinach and carrot; fruits of the group consisting of peach, persimmon, grape and apple; and flowers of the group consisting of tulip and cosmos.

14. The method of claim 9 wherein the plant is selected from rice, wheat, corn and soybean.

15. Use of a 24-epibrassinolide and a nontoxic salt of choline for increasing the quality and quantity of fruits and flowers of a plant in horticulture and agriculture.



European Patent
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EUROPEAN SEARCH REPORT

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Application number

EP 86 11 3368

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	US-A-4 346 226 (M.J.THOMPSON et al.)	1-15	A 01 N 49/00 // (A 01 N 49/00 A 01 N 33:12)
A	GB-A-2 059 412 (B.KESSLER) & US-A-4 309 205 (Cat. D)	1-15	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			A 01 N
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 13-01-1987	Examiner DECORTE D.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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